

What is claimed is:

1. An isolated nucleic acid encoding a mammalian NPFF receptor.
2. The nucleic acid of claim 1, wherein the nucleic acid is DNA.
3. The DNA of claim 2, wherein the DNA is cDNA.
4. The DNA of claim 2, wherein the DNA is genomic DNA.
5. The nucleic acid of claim 1, wherein the nucleic acid is RNA.
6. The nucleic acid of claim 1, wherein the mammalian NPFF receptor is a NPFF1 receptor.
7. The nucleic acid of claim 6, wherein the mammalian NPFF1 receptor is a rat NPFF1 receptor.
8. The nucleic acid of claim 6, wherein the mammalian NPFF1 receptor is a human NPFF1 receptor.
9. The nucleic acid of claim 1, wherein the mammalian NPFF receptor is a NPFF2 receptor.
10. The nucleic acid of claim 9, wherein the mammalian NPFF2 receptor is a human NPFF2 receptor.
11. The nucleic acid of claim 9, wherein the mammalian NPFF2 receptor is a rat NPFF2 receptor.
12. The nucleic acid of claim 7, wherein the rat NPFF1 receptor has an amino acid sequence identical to that encoded by the plasmid pEXJ-rNPFF1 (ATCC Accession No. 203184)

13. The nucleic acid of claim 7, wherein the rat NPFF1 receptor has an amino acid sequence identical to the amino acid sequence shown in Figure 2 (SEQ ID NO: 2).
14. The nucleic acid of claim 8, wherein the human NPFF1 receptor has an amino acid sequence identical to that encoded by plasmid pWE15-hNPFF1 (ATCC Accession No. 203183).
15. The nucleic acid of claim 8, wherein the human NPFF1 receptor has an amino acid sequence identical to the amino acid sequence shown in Figure 5 SEQ ID NO: 4).
16. The nucleic acid of claim 8, wherein the human NPFF1 receptor has an amino acid sequence identical to that encoded by plasmid pcDNA3.1-hNPFF1 (ATCC Accession No. 203605).
17. The nucleic acid of claim 8, wherein the human NPFF1 receptor has an amino acid sequence identical to the amino acid sequence shown in Figure 12 (SEQ ID NO: 8).
18. The nucleic acid of claim 10, wherein the human NPFF2 receptor has an amino acid sequence identical to that encoded by plasmid pcDNA3.1-hNPFF2b (ATCC Accession No. 203255).
19. The nucleic acid of claim 10, wherein the human NPFF2 receptor has an amino acid sequence identical to the amino acid sequence shown in Figure 8 (SEQ ID NO: 6).
20. The nucleic acid of claim 11, wherein the rat NPFF2 receptor has an amino acid sequence identical to

that encoded by plasmid pcDNA3.1-rNPFF2-f (Patent Deposit Designation No. PTA-535).

- 5 21. The nucleic acid of claim 11, wherein the rat NPFF2 receptor has an amino acid sequence identical to the amino acid sequence shown in Figures 23A-B (SEQ ID NO: 44).
- 10 22. The nucleic acid of claim 1, wherein the nucleic acid (a) hybridizes to a nucleic acid having the defined sequence shown in Figure 1 (SEQ ID NO: 1) under low stringency conditions or a sequence complementary thereto and (b) is further characterized by its ability to cause a change in the pH of a culture of CHO cells when a NPFF peptide is added to the culture and the CHO cells express the nucleic acid which hybridized to the nucleic acid having the defined sequence of its complement.
- 15 23. The nucleic acid of claim 1, wherein the nucleic acid (a) hybridizes to a nucleic acid having the defined sequence shown in Figure 4 (SEQ ID NO: 3) under low stringency conditions or a sequence complementary thereto and (b) is further characterized by its ability to cause a change in the pH of a culture of CHO cells when a NPFF peptide is added to the culture and the CHO cells express the nucleic acid which hybridized to the nucleic acid having the defined sequence or its complement.
- 20 24. The nucleic acid of claim 1, wherein the nucleic acid (a) hybridizes to a nucleic acid having the defined sequence shown in Figure 7 (SEQ ID NO: 5) under low stringency conditions or a sequence complementary thereto and (b) is further
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characterized by its ability to cause a change in the pH of a culture of CHO cells when a NPFF peptide is added to the culture and the CHO cells express the nucleic acid which hybridized to the nucleic acid having the defined sequence or its complement.

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25. The nucleic acid of claim 1, wherein the nucleic acid (a) hybridizes to a nucleic acid having the defined sequence shown in Figure 11 (SEQ ID NO: 7) under low stringency conditions or a sequence complementary thereto and (b) is further characterized by its ability to cause a change in the pH of a culture of CHO cells when a NPFF peptide is added to the culture and the CHO cells express the nucleic acid which hybridized to the nucleic acid having the defined sequence or its complement.

26. The nucleic acid of claim 1, wherein the nucleic acid (a) hybridizes to a nucleic acid having the defined sequence shown in Figures 22A-C (SEQ ID NO: 43) under low stringency conditions or a sequence complementary thereto and (b) is further characterized by its ability to cause a change in the pH of a culture of CHO cells when a NPFF peptide is added to the culture and the CHO cells express the nucleic acid which hybridized to the nucleic acid having the defined sequence or its complement.

27. A purified mammalian NPFF receptor protein.

28. The purified mammalian NPFF receptor protein of claim 27, wherein the NPFF receptor protein is a NPFF1 receptor protein.

29. The purified mammalian NPFF receptor protein of claim 27, wherein the NPFF receptor protein is a NPFF2 receptor protein.
- 5 30. The purified mammalian NPFF1 receptor protein of claim 28, wherein the NPFF1 receptor protein is a rat NPFF1 receptor protein.
- 10 31. The purified mammalian NPFF1 receptor protein of claim 28, wherein the NPFF1 receptor protein is a human NPFF1 receptor protein.
- 15 32. The purified mammalian NPFF2 receptor protein of claim 29, wherein the NPFF2 receptor protein is a human NPFF2 receptor protein.
- 20 33. The purified mammalian NPFF2 receptor protein of claim 29, wherein the NPFF2 receptor protein is a rat NPFF2 receptor protein.
- 25 34. A vector comprising the nucleic acid of claim 1.
- 35 35. A vector comprising the nucleic acid of claim 6.
36. A vector comprising the nucleic acid of claim 9.
37. A vector comprising the nucleic acid of any of claims 22, 23, 24, or 25.
- 30 38. A vector of any of claims 22, 23, 24, 25, 34, 35, or 36 adapted for expression in a cell which comprises the regulatory elements necessary for expression of the nucleic acid in the cell operatively linked to the nucleic acid encoding the receptor so as to permit expression thereof,
- 35 wherein the cell is a bacterial, amphibian, yeast, insect or mammalian cell.

39. The vector of claim 38, wherein the vector is a baculovirus.
- 5 40. The vector of claim 34, wherein the vector is a plasmid.
41. The plasmid of claim 40 designated pEXJ-rNPFF1 (ATCC Accession No. 203184).
- 10 42. The plasmid of claim 40 designated pWE15-hNPFF1 (ATCC Accession No. 203183).
43. The plasmid of claim 40 designated pCDNA3.1-hNPFF2b (ATCC Accession No. 203255).
- 15 44. The plasmid of claim 40 designated pCDNA3.1-hNPFF1 (ATCC Accession No. 203505).
- 20 45. The plasmid of claim 40 designated pCDNA3.1-rNPFF2-f (Patent Deposit Designation No. PTA-535).
46. A cell comprising the vector of claim 38.
- 25 47. A cell of claim 46, wherein the cell is a non-mammalian cell.
48. A cell of claim 47, wherein the non-mammalian cell is a *Xenopus* oocyte cell or a *Xenopus* melanophore cell.
- 30 49. A cell of claim 46, wherein the cell is a mammalian cell.
- 35 50. A mammalian cell of claim 49, wherein the cell is a COS-7 cell, a 293 human embryonic kidney cell, a NIH-3T3 cell, a LM(tk-) cell, a mouse Y1 cell, or a CHO cell.

51. An insect cell comprising the vector of claim 38.
52. An insect cell of claim 51, wherein the insect cell is an Sf9 cell, an Sf21 cell or a Trichoplusia ni 5B1-4 cell.
53. A membrane preparation isolated from the cell of any of claims 46, 47, 49, 50, 51 or 52.
54. A nucleic acid probe comprising at least 15 nucleotides, which probe specifically hybridizes with a nucleic acid encoding a mammalian NPFF receptor, wherein the probe has a unique sequence corresponding to a sequence present within one of the two strands of the nucleic acid encoding the mammalian NPFF1 receptor and contained in plasmid pEXJ-rNPFF1 (ATCC Accession No. 203184), plasmid pWE15-hNPFF1 (ATCC Accession No. 203183), plasmid pCDNA3.1-hNPFF2b (ATCC Accession No. 203255), plasmid pcDNA3.1-hNPFF1 (ATCC Accession No. 203605), or pcDNA3.1-rNPFF2-f (Patent Deposit Designation No. PTA-535).
55. A nucleic acid probe comprising at least 15 nucleotides, which probe specifically hybridizes with a nucleic acid encoding a mammalian NPFF receptor, wherein the probe has a unique sequence corresponding to a sequence present within (a) the nucleic acid sequence shown in Figure 1 (SEQ ID NO: 1) or (b) the reverse complement thereto.
56. A nucleic acid probe comprising at least 15 nucleotides, which probe specifically hybridizes with a nucleic acid encoding a mammalian NPFF receptor, wherein the probe has a unique sequence corresponding to a sequence present within (a) the nucleic acid sequence shown in Figure 4 (SEQ ID NO:

3) or (b) the reverse complement thereto.

57. A nucleic acid probe comprising at least 15 nucleotides, which probe specifically hybridizes with a nucleic acid encoding a mammalian NPFF receptor, wherein the probe has a unique sequence corresponding to a sequence present within (a) the nucleic acid sequence shown in Figure 7 (SEQ ID NO: 5) or (b) the reverse complement thereto.

58. A nucleic acid probe comprising at least 15 nucleotides, which probe specifically hybridizes with a nucleic acid encoding a mammalian NPFF receptor, wherein the probe has a unique sequence corresponding to a sequence present within (a) the nucleic acid sequence shown in Figure 11 (SEQ ID NO: 7) or (b) the reverse complement thereto.

59. A nucleic acid probe comprising at least 15 nucleotides, which probe specifically hybridizes with a nucleic acid encoding a mammalian NPFF receptor, wherein the probe has a unique sequence corresponding to a sequence present within (a) the nucleic acid sequence shown in Figures 22A-C (SEQ ID NO: 43) or (b) the reverse complement thereto.

60. The nucleic acid probe of claim 55, 56, 57, or 58, wherein the nucleic acid is DNA.

61. The nucleic acid probe of claim 55, 56, 57, or 58, wherein the nucleic acid is RNA.

62. An antisense oligonucleotide having a sequence capable of specifically hybridizing to the RNA of claim 5, so as to prevent translation of the RNA.

63. An antisense oligonucleotide having a sequence

capable of specifically hybridizing to the genomic DNA of claim 4, so as to prevent transcription of the genomic DNA.

- 5 64. An antisense oligonucleotide of claim 62 or 63, wherein the oligonucleotide comprises chemically modified nucleotides or nucleotide analogues.
- 10 65. An antibody capable of binding to a mammalian NPFF receptor encoded by the nucleic acid of claim 1.
- 15 66. An antibody of claim 65, wherein the mammalian NPFF receptor is a human NPFF1 receptor.
- 15 67. An antibody of claim 65, wherein the mammalian NPFF receptor is a rat NPFF1 receptor.
- 20 68. An antibody of claim 65, wherein the mammalian NPFF receptor is a human NPFF2 receptor.
- 20 69. An antibody of claim 65, wherein the mammalian NPFF receptor is a rat NPFF2 receptor.
- 25 70. An agent capable of competitively inhibiting the binding of the antibody of claim 65 to a mammalian NPFF receptor.
- 30 71. An antibody of claim 65, wherein the antibody is a monoclonal antibody or antisera.
- 35 72. A pharmaceutical composition comprising (a) an amount of the oligonucleotide of claim 62 capable of passing through a cell membrane and effective to reduce expression of a mammalian NPFF receptor and (b) a pharmaceutically acceptable carrier capable of passing through the cell membrane.

73. A pharmaceutical composition of claim 72, wherein the oligonucleotide is coupled to a substance which inactivates mRNA.
- 5 74. A pharmaceutical composition of claim 73, wherein the substance which inactivates mRNA is a ribozyme.
75. A pharmaceutical composition of claim 73, wherein the pharmaceutically acceptable carrier comprises a structure which binds to a mammalian NPFF receptor on a cell capable of being taken up by the cells after binding to the structure.
- 10 76. A pharmaceutical composition of claim 75, wherein the pharmaceutically acceptable carrier is capable of binding to a mammalian NPFF receptor which is specific for a selected cell type.
- 15 77. A pharmaceutical composition which comprises an amount of the antibody of claim 65 effective to block binding of a ligand to a human NPFF receptor and a pharmaceutically acceptable carrier.
- 20 78. A transgenic, nonhuman mammal expressing DNA encoding a mammalian NPFF receptor of claim 1.
- 25 79. A transgenic, nonhuman mammal comprising a homologous recombination knockout of the native mammalian NPFF receptor.
- 30 80. A transgenic, nonhuman mammal whose genome comprises antisense DNA complementary to the DNA encoding a mammalian NPFF receptor of claim 1 so placed within the genome as to be transcribed into antisense mRNA which is complementary to mRNA encoding the mammalian NPFF receptor and which hybridizes to mRNA encoding the mammalian NPFF
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receptor, thereby reducing its translation.

- 5 81. The transgenic, nonhuman mammal of claim 78 or 79,
wherein the DNA encoding the mammalian NPFF
receptor additionally comprises an inducible
promoter.
- 10 82. The transgenic, nonhuman mammal of claim 78 or 79,
wherein the DNA encoding the mammalian NPFF
receptor additionally comprises tissue specific
regulatory elements.
- 15 83. A transgenic, nonhuman mammal of claim 78, 79, or
80, wherein the transgenic, nonhuman mammal is a
mouse.
- 20 84. A process for identifying a chemical compound which
specifically binds to a mammalian NPFF receptor
which comprises contacting cells containing DNA
encoding and expressing on their cell surface the
mammalian NPFF receptor, wherein such cells do not
normally express the mammalian NPFF receptor, with
the compound under conditions suitable for binding,
and detecting specific binding of the chemical
25 compound to the mammalian NPFF receptor.
- 30 85. A process for identifying a chemical compound which
specifically binds to a mammalian NPFF receptor
which comprises contacting a membrane preparation
from cells containing DNA encoding and expressing
on their cell surface the mammalian NPFF receptor,
wherein such cells do not normally express the
mammalian NPFF receptor, with the compound under
conditions suitable for binding, and detecting
35 specific binding of the chemical compound to the
mammalian NPFF receptor.

86. The process of claim 84 or 85, wherein the mammalian NPFF receptor is a human NPFF1 receptor.
- 5 87. The process of claim 84 or 85, wherein the mammalian NPFF receptor is a human NPFF2 receptor.
- 10 88. The process of claim 84 or 85, wherein the mammalian NPFF receptor has substantially the same amino acid sequence as the human NPFF1 receptor encoded by plasmid pWE15-hNPFF1 (ATCC Accession No. 203183).
- 15 89. The process of claim 84 or 85, wherein the mammalian NPFF receptor has substantially the same amino acid sequence as the human NPFF1 receptor encoded by plasmid pCDNA3.1-hNPFF1 (ATCC Accession No. 203605).
- 20 90. The process of claim 84 or 85, wherein the mammalian NPFF receptor has substantially the same amino acid sequence as the human NPFF2 receptor encoded by plasmid pCDNA3.1-hNPFF2b (ATCC Accession No. 203255).
- 25 91. The process of claim 84 or 85, wherein the mammalian NPFF receptor has substantially the same amino acid sequence as that shown in Figure 5 (SEQ ID NO: 4).
- 30 92. The process of claim 84 or 85, wherein the mammalian NPFF receptor has the amino acid sequence shown in Figure 5 (SEQ ID NO: 4).
- 35 93. The process of claim 84 or 85, wherein the mammalian NPFF receptor has substantially the same amino acid sequence as that shown in Figure 8 (SEQ ID NO: 6).

94. The process of claim 84 or 85, wherein the mammalian NPFF receptor has the same amino acid sequence shown in Figure 8 (SEQ ID NO: 6).
- 5 95. The process of claim 84 or 85, wherein the mammalian NPFF receptor has substantially the same amino acid sequence as that shown in Figure 12 (SEQ ID NO: 8).
- 10 96. The process of claim 84 or 85, wherein the mammalian NPFF receptor has the same amino acid sequence shown in Figure 12 (SEQ ID NO: 8).
- 15 97. The process of claim 84 or 85, wherein the compound is not previously known to bind to a mammalian NPFF receptor.
98. A compound identified by the process of claim 97.
- 20 99. A process of claim 84 or 85, wherein the cell is an insect cell.
100. The process of claim 84 or 85, wherein the cell is a mammalian cell.
- 25 101. The process of claim 100, wherein the cell is nonneuronal in origin.
102. The process of claim 101, wherein the nonneuronal cell is a COS-7 cell, 293 human embryonic kidney cell, a CHO cell, a NIH-3T3 cell, a mouse Y1 cell, or a LM(tk-) cell.
- 30 103. A process of claim 100, wherein the compound is a compound not previously known to bind to a mammalian NPFF receptor.
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104. A compound identified by the process of claim 103.

5 105. A process involving competitive binding for identifying a chemical compound which specifically binds to a mammalian NPFF receptor which comprises separately contacting cells expressing on their cell surface the mammalian NPFF receptor, wherein such cells do not
10 normally express the mammalian NPFF receptor, with both the chemical compound and a second chemical compound known to bind to the receptor, and with only the second chemical compound, under conditions suitable for binding
15 of both compounds, and detecting specific binding of the chemical compound to the mammalian NPFF receptor, a decrease in the binding of the second chemical compound to the mammalian NPFF receptor in the presence of the
20 chemical compound indicating that the chemical compound binds to the mammalian NPFF receptor.

25 106. A process involving competitive binding for identifying a chemical compound which specifically binds to a mammalian NPFF receptor which comprises separately contacting a membrane preparation from cells expressing on
30 their cell surface the mammalian NPFF receptor, wherein such cells do not normally express the mammalian NPFF receptor, with both the chemical compound and a second chemical compound known to bind to the receptor, and with only the
35 second chemical compound, under conditions suitable for binding of both compounds, and detecting specific binding of the chemical compound to the mammalian NPFF receptor, a decrease in the binding of the second chemical

compound to the mammalian NPFF receptor in the presence of the chemical compound indicating that the chemical compound binds to the mammalian NPFF receptor.

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107. A process of claim 105 or 106, wherein the mammalian NPFF receptor is a human NPFF1 receptor.

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108. A process of claim 105 or 106, wherein the mammalian NPFF receptor is a human NPFF2 receptor.

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109. The process of claim 105 or 106, wherein the cell is an insect cell.

110. The process of claim 105 or 106, wherein the cell is a mammalian cell.

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111. The process of claim 110, wherein the cell is nonneuronal in origin.

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112. The process of claim 111, wherein the nonneuronal cell is a COS-7 cell, 293 human embryonic kidney cell, a CHO cell, a NIH-3T3 cell, a mouse Y1 cell, or a LM(tk-) cell.

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113. The process of claim 112, wherein the compound is not previously known to bind to a mammalian NPFF receptor.

114. A compound identified by the process of claim 113.

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115. A method of screening a plurality of chemical compounds not known to bind to a mammalian NPFF receptor to identify a compound which

specifically binds to the mammalian NPFF receptor, which comprises

- 5 (a) contacting cells transfected with and expressing DNA encoding the mammalian NPFF receptor with a compound known to bind specifically to the mammalian NPFF receptor;
- 10 (b) contacting the preparation of step (a) with the plurality of compounds not known to bind specifically to the mammalian NPFF receptor, under conditions permitting binding of compounds known to bind to the
15 mammalian NPFF receptor;
- (c) determining whether the binding of the compound known to bind to the mammalian NPFF receptor is reduced in the presence
20 of any compound within the plurality of compounds, relative to the binding of the compound in the absence of the plurality of compounds; and if so
- 25 (d) separately determining the binding to the mammalian NPFF receptor of compounds included in the plurality of compounds, so as to thereby identify the compound which
30 specifically binds to the mammalian NPFF receptor.

116. A method of screening a plurality of chemical compounds not known to bind to a mammalian NPFF receptor to identify a compound which
35 specifically binds to the mammalian NPFF receptor, which comprises

- 5 (a) contacting a membrane preparation from cells transfected with and expressing DNA encoding the mammalian NPFF receptor with the plurality of compounds not known to bind specifically to the mammalian NPFF receptor under conditions permitting binding of compounds known to bind to the mammalian NPFF receptor;
- 10 (b) determining whether the binding of a compound known to bind to the mammalian NPFF receptor is reduced in the presence of any compound within the plurality of compounds, relative to the binding of the compound in the absence of the plurality of compounds; and if so
- 15 (c) separately determining the binding to the mammalian NPFF receptor of compounds included in the plurality of compounds, so as to thereby identify the compound which specifically binds to the mammalian NPFF receptor.
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- 25 117. A method of claim 115 or 116, wherein the mammalian NPFF receptor is a human NPFF1 receptor.
- 30 118. A method of claim 115 or 116, wherein the mammalian NPFF receptor is a human NPFF2 receptor.
- 35 119. A method of claim 115 or 116, wherein the cell is a mammalian cell.
120. A method of claim 119, wherein the mammalian cell is non-neuronal in origin.

121. The method of claim 120, wherein the non-neuronal cell is a COS-7 cell, a 293 human embryonic kidney cell, a LM(tk-) cell, a CHO cell, a mouse Y1 cell, or an NIH-3T3 cell.

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122. A method of detecting expression of a mammalian NPFF receptor by detecting the presence of mRNA coding for the mammalian NPFF receptor which comprises obtaining total mRNA from the cell and contacting the mRNA so obtained with the nucleic acid probe of any of claims 54, 55, 56, 57, or 58 under hybridizing conditions, detecting the presence of mRNA hybridizing to the probe, and thereby detecting the expression of the mammalian NPFF receptor by the cell.

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123. A method of detecting the presence of a mammalian NPFF receptor on the surface of a cell which comprises contacting the cell with the antibody of claim 65 under conditions permitting binding of the antibody to the receptor, detecting the presence of the antibody bound to the cell, and thereby detecting the presence of the mammalian NPFF receptor on the surface of the cell.

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124. A method of determining the physiological effects of varying levels of activity of mammalian NPFF receptors which comprises producing a transgenic, nonhuman mammal of claim 81 whose levels of mammalian NPFF receptor activity are varied by use of an inducible promoter which regulates mammalian NPFF receptor expression.

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125. A method of determining the physiological effects of varying levels of activity of

mammalian NPFF receptors which comprises producing a panel of transgenic, nonhuman mammals of claim 81 each expressing a different amount of mammalian NPFF receptor.

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126. A method for identifying an antagonist capable of alleviating an abnormality wherein the abnormality is alleviated by decreasing the activity of a mammalian NPFF receptor comprising administering a compound to the transgenic, nonhuman mammal of claim 78, 81, 82, or 83, and determining whether the compound alleviates the physical and behavioral abnormalities displayed by the transgenic, nonhuman mammal as a result of overactivity of a mammalian NPFF receptor, the alleviation of the abnormality identifying the compound as an antagonist.

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127. The method of claim 126, wherein the mammalian NPFF receptor is a human NPFF1 receptor.

128. The method of claim 126, wherein the mammalian NPFF receptor is a human NPFF2 receptor.

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129. An antagonist identified by the method of claim 126.

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130. A pharmaceutical composition comprising an antagonist of claim 129 and a pharmaceutically acceptable carrier.

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131. A method of treating an abnormality in a subject wherein the abnormality is alleviated by decreasing the activity of a mammalian NPFF receptor which comprises administering to the subject an effective amount of the

pharmaceutical composition of claim 130, thereby treating the abnormality.

- 5 132. A method for identifying an agonist capable of alleviating an abnormality in a subject wherein the abnormality is alleviated by increasing the activity of a mammalian NPFF receptor comprising administering a compound to the transgenic, nonhuman mammal of claim 78, 81, 10 82, or 83, and determining whether the compound alleviates the physical and behavioral abnormalities displayed by the transgenic, nonhuman mammal, the alleviation of the abnormality identifying the compound as an agonist.
- 15 133. The method of claim 132, wherein the mammalian NPFF receptor is a human NPFF1 receptor.
- 20 134. The method of claim 132, wherein the mammalian NPFF receptor is a human NPFF2 receptor.
- 25 135. An agonist identified by the method of claim 132.
- 30 136. A pharmaceutical composition comprising an agonist identified by the method of claim 135 and a pharmaceutically acceptable carrier.
- 35 137. A method of treating an abnormality in a subject wherein the abnormality is alleviated by increasing the activity of a mammalian NPFF receptor which comprises administering to the subject an effective amount of the pharmaceutical composition of claim 136, thereby treating the abnormality.

138. A method for diagnosing a predisposition to a disorder associated with the activity of a specific mammalian allele which comprises:

- 5 (a) obtaining DNA of subjects suffering from the disorder;
- (b) performing a restriction digest of the DNA with a panel of restriction enzymes;
- 10 (c) electrophoretically separating the resulting DNA fragments on a sizing gel;
- (d) contacting the resulting gel with a nucleic acid probe capable of specifically hybridizing with a unique sequence included within the sequence of a nucleic acid molecule encoding a mammalian NPFF receptor and labeled with a detectable marker;
- 15 (e) detecting labeled bands which have hybridized to the DNA encoding a mammalian NPFF1 receptor of claim 1 labeled with a detectable marker to create a unique band pattern specific to the DNA of subjects suffering from the disorder;
- 25 (f) preparing DNA obtained for diagnosis by steps (a) - (e); and
- 30 (g) comparing the unique band pattern specific to the DNA of subjects suffering from the disorder from step (e) and the DNA obtained for diagnosis from step (f) to determine whether the patterns are the same or different and to diagnose thereby
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predisposition to the disorder if the patterns are the same.

139. The method of claim 138, wherein a disorder
5 associated with the activity of a specific
mammalian allele is diagnosed.

140. A method of preparing the purified mammalian
NPFF receptor of claim 27 which comprises:

10 (a) culturing cells which express the
mammalian NPFF receptor;

15 (b) recovering the mammalian NPFF receptor
from the cells; and

(c) purifying the mammalian NPFF receptor so
recovered.

20 141. A method of preparing the purified mammalian
NPFF receptor of claim 27 which comprises:

25 (a) inserting a nucleic acid encoding the
mammalian NPFF receptor into a suitable
vector;

(b) introducing the resulting vector into a
suitable host cell;

30 (c) placing the resulting cell in suitable
condition permitting the production of the
mammalian NPFF receptor;

35 (d) recovering the mammalian NPFF receptor
produced by the resulting cell; and

(e) isolating and/or purifying the mammalian

NPFF receptor so recovered.

142. A process for determining whether a chemical compound is a mammalian NPFF receptor agonist which comprises contacting cells transfected with and expressing DNA encoding the mammalian NPFF receptor with the compound under conditions permitting the activation of the mammalian NPFF receptor, and detecting an increase in mammalian NPFF receptor activity, so as to thereby determine whether the compound is a mammalian NPFF receptor agonist.
143. A process for determining whether a chemical compound is a mammalian NPFF receptor antagonist which comprises contacting cells transfected with and expressing DNA encoding the mammalian NPFF receptor with the compound in the presence of a known mammalian NPFF receptor agonist, under conditions permitting the activation of the mammalian NPFF receptor, and detecting a decrease in mammalian NPFF receptor activity, so as to thereby determine whether the compound is a mammalian NPFF receptor antagonist.
144. A process of claim 142 or 143, wherein the mammalian NPFF receptor is a human NPFF1 receptor.
145. A process of claim 142 or 143, wherein the mammalian NPFF receptor is a human NPFF2 receptor.
146. A pharmaceutical composition which comprises an amount of a mammalian NPFF receptor agonist determined by the process of claim 142

effective to increase activity of a mammalian NPFF receptor and a pharmaceutically acceptable carrier.

5 147. A pharmaceutical composition of claim 146,
 wherein the mammalian NPFF receptor agonist is
 not previously known.

10 148. A pharmaceutical composition which comprises an
 amount of a mammalian NPFF receptor antagonist
 determined by the process of claim 143
 effective to reduce activity of a mammalian
 NPFF receptor and a pharmaceutically acceptable
 carrier.

15 149. A pharmaceutical composition of claim 148,
 wherein the mammalian NPFF receptor antagonist
 is not previously known.

20 150. A process for determining whether a chemical
 compound specifically binds to and activates a
 mammalian NPFF receptor, which comprises
 contacting cells producing a second messenger
 response and expressing on their cell surface
25 the mammalian NPFF receptor, wherein such cells
 do not normally express the mammalian NPFF
 receptor, with the chemical compound under
 conditions suitable for activation of the
 mammalian NPFF receptor, and measuring the
30 second messenger response in the presence and
 in the absence of the chemical compound, a
 change in the second messenger response in the
 presence of the chemical compound indicating
 that the compound activates the mammalian NPFF
35 receptor.

151. The process of claim 150, wherein the second

messenger response comprises chloride channel activation and the change in second messenger is an increase in the level of inward chloride current.

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152. A process for determining whether a chemical compound specifically binds to and inhibits activation of a mammalian NPFF receptor, which comprises separately contacting cells producing a second messenger response and expressing on their cell surface the mammalian NPFF receptor, wherein such cells do not normally express the mammalian NPFF receptor, with both the chemical compound and a second chemical compound known to activate the mammalian NPFF receptor, and with only the second chemical compound, under conditions suitable for activation of the mammalian NPFF receptor, and measuring the second messenger response in the presence of only the second chemical compound and in the presence of both the second chemical compound and the chemical compound, a smaller change in the second messenger response in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound indicating that the chemical compound inhibits activation of the mammalian NPFF receptor.

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153. The process of claim 152, wherein the second messenger response comprises chloride channel activation and the change in second messenger response is a smaller increase in the level of inward chloride current in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound

154. A process of any of claims 150, 151, 152, or 153, wherein the mammalian NPFF receptor is a human NPFF1 receptor.
- 5 155. A process of any of claims 150, 151, 152, or 153, wherein the mammalian NPFF receptor is a human NPFF2 receptor.
- 10 156. The process of any of claims 150, 151, 152, or 153, wherein the cell is an insect cell.
157. The process of any of claims 150, 151, 152, or 153, wherein the cell is a mammalian cell.
- 15 158. The process of claim 157, wherein the mammalian cell is nonneuronal in origin.
159. The process of claim 158, wherein the nonneuronal cell is a COS-7 cell, CHO cell, 293 human embryonic kidney cell, NIH-3T3 cell or LM(tk-) cell.
- 20 160. The process of claim 150, 151, 152, or 153, wherein the compound is not previously known to bind to a mammalian NPFF receptor.
- 25 161. A compound determined by the process of claim 160.
- 30 162. A pharmaceutical composition which comprises an amount of a mammalian NPFF receptor agonist determined by the process of claim 150 or 151 effective to increase activity of a mammalian NPFF receptor and a pharmaceutically acceptable carrier.
- 35 163. A pharmaceutical composition of claim 162,

wherein the mammalian NPFF receptor agonist is not previously known.

- 5 164. A pharmaceutical composition which comprises an amount of a mammalian NPFF receptor antagonist determined by the process of claim 152 or 153 effective to reduce activity of a mammalian NPFF receptor and a pharmaceutically acceptable carrier.
- 10 165. A pharmaceutical composition of claim 164, wherein the mammalian NPFF receptor antagonist is not previously known.
- 15 166. A method of screening a plurality of chemical compounds not known to activate a mammalian NPFF receptor to identify a compound which activates the mammalian NPFF receptor which comprises:
- 20 (a) contacting cells transfected with and expressing the mammalian NPFF receptor with the plurality of compounds not known to activate the mammalian NPFF receptor, under conditions permitting activation of the mammalian NPFF receptor;
- 25 (b) determining whether the activity of the mammalian NPFF receptor is increased in the presence of the compounds; and if so
- 30 (c) separately determining whether the activation of the mammalian NPFF receptor is increased by each compound included in the plurality of compounds, so as to thereby identify the compound which activates the mammalian NPFF receptor.
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167. A method of claim 166, wherein the mammalian NPFF receptor is a human NPFF1 receptor.

168. A method of claim 166, wherein the mammalian NPFF receptor is a human NPFF2 receptor.

169. A method of screening a plurality of chemical compounds not known to inhibit the activation of a mammalian NPFF receptor to identify a compound which inhibits the activation of the mammalian NPFF receptor, which comprises:

(a) contacting cells transfected with and expressing the mammalian NPFF receptor with the plurality of compounds in the presence of a known mammalian NPFF receptor agonist, under conditions permitting activation of the mammalian NPFF receptor;

(b) determining whether the activation of the mammalian NPFF receptor is reduced in the presence of the plurality of compounds, relative to the activation of the mammalian NPFF receptor in the absence of the plurality of compounds; and if so

(c) separately determining the inhibition of activation of the mammalian NPFF receptor for each compound included in the plurality of compounds, so as to thereby identify the compound which inhibits the activation of the mammalian NPFF receptor.

170. A method of claim 169, wherein the mammalian NPFF receptor is a human NPFF1 receptor.

171. A method of claim 169, wherein the mammalian NPFF receptor is a human NPFF2 receptor.
- 5 172. A method of any of claims 166, 167, 168, 169, 162, or 163, wherein the cell is a mammalian cell.
- 10 173. A method of claim 172, wherein the mammalian cell is non-neuronal in origin.
- 15 174. The method of claim 173, wherein the non-neuronal cell is a COS-7 cell, a 293 human embryonic kidney cell, a LM(tk-) cell or an NIH-3T3 cell.
- 20 175. A pharmaceutical composition comprising a compound identified by the method of claim 166 or 167 effective to increase mammalian NPFF receptor activity and a pharmaceutically acceptable carrier.
- 25 176. A pharmaceutical composition comprising a compound identified by the method of claim 169 or 170 effective to decrease mammalian NPFF receptor activity and a pharmaceutically acceptable carrier.
- 30 177. A method of treating an abnormality in a subject wherein the abnormality is alleviated by increasing the activity of a mammalian NPFF receptor which comprises administering to the subject an amount of a compound which is a mammalian NPFF receptor agonist effective to treat the abnormality.
- 35 178. A method of claim 177, wherein the abnormality is a lower urinary tract disorder, a regulation

of a steroid hormone disorder, an epinephrine
release disorder, a gastrointestinal disorder,
irritable bowel syndrome, a cardiovascular
disorder, an electrolyte balance disorder,
5 diuresis, hypertension, hypotension, diabetes,
hypoglycemia, a respiratory disorder, asthma,
a reproductive function disorder, an immune
disorder, an endocrine disorder, a
musculoskeletal disorder, a neuroendocrine
10 disorder, a cognitive disorder, a memory
disorder, a sensory modulation and transmission
disorder, a motor coordination disorder, a
sensory integration disorder, a motor
integration disorder, a dopaminergic function
15 disorder, a serotonergic function disorder, an
appetite disorder, obesity, a sensory
transmission disorder, an olfaction disorder,
a sympathetic innervation disorder, pain,
psychotic behavior, morphine tolerance,
20 nicotine addiction, opiate addiction, affective
disorder, or migraine.

179. A method of treating an abnormality in a
subject wherein the abnormality is alleviated
25 by decreasing the activity of a mammalian NPFF
receptor which comprises administering to the
subject an amount of a compound which is a
mammalian NPFF receptor antagonist effective to
treat the abnormality.

30
180. A method of claim 179, wherein the abnormality
is a lower urinary tract disorder, a regulation
of steroid hormone disorder, an epinephrine
release disorder, a gastrointestinal disorder,
35 irritable bowel syndrome, a cardiovascular
disorder, an electrolyte balance disorder,
diuresis, hypertension, hypotension, diabetes,

hypoglycemia, a respiratory disorder, asthma,
a reproductive function disorder, an immune
disorder, an endocrine disorder, a
musculoskeletal disorder, a neuroendocrine
disorder, a cognitive disorder, a memory
disorder, a sensory modulation and transmission
disorder, a motor coordination disorder, a
sensory integration disorder, a motor
integration disorder, a dopaminergic function
disorder, a serotonergic function disorder, an
appetite disorder, obesity, a sensory
transmission disorder, an olfaction disorder,
a sympathetic innervation disorder, pain,
psychotic behavior, morphine tolerance,
nicotine addiction, opiate addiction, affective
disorder, or migraine.

181. A process for making a composition of matter
which specifically binds to a mammalian NPFF
receptor which comprises identifying a chemical
compound using the process of any of claims 84,
85, 105, 106, 115, or 116 and then synthesizing
the chemical compound or a novel structural and
functional analog or homolog thereof.

182. A process for making a composition of matter
which specifically binds to a mammalian NPFF
receptor which comprises identifying a chemical
compound using the process of any of claims
134, 150, or 166 and then synthesizing the
chemical compound or a novel structural and
functional analog or homolog thereof.

183. A process for making a composition of matter
which specifically binds to a mammalian NPFF
receptor which comprises identifying a chemical
compound using the process of any of claims

143, 152, 169 and then synthesizing the chemical compound or a novel structural and functional analog or homolog thereof.

5 184. The process of any of claims 181, 182, or 183, wherein the mammalian NPFF receptor is a human NPFF1 receptor.

10 185. The process of any of claims 181, 182, or 183, wherein the mammalian NPFF receptor is a human NPFF2 receptor.

15 186. A process for preparing a pharmaceutical composition which comprises admixing a pharmaceutically acceptable carrier and a pharmaceutically acceptable amount of a chemical compound identified by the process of any of claims 84, 85, 105, 106, 115, or 116 or a novel structural and functional analog or homolog thereof.

20 187. A process for preparing a pharmaceutical composition which comprises admixing a pharmaceutically acceptable carrier and a pharmaceutically acceptable amount of a chemical compound identified by the process of any of claims 142, 150, or 166 or a novel structural and functional analog or homolog thereof.

25 188. A process for preparing a pharmaceutical composition which comprises admixing a pharmaceutically acceptable carrier and a pharmaceutically acceptable amount of a chemical compound identified by the process of any of claims 143, 152, or 169 or a novel structural and functional analog or homolog

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thereof.

- 5 189. The process of any of claims 186, 187, or 188, wherein the mammalian NPFF receptor is a human NPFF1 receptor.
- 10 190. The process of any of claims 186, 187, or 188, wherein the mammalian NPFF receptor is a human NPFF2 receptor.
- 15 191. A method of claim 117, wherein the cell is a mammalian cell.
- 20 192. The method of claim 191, wherein the mammalian cell is non-neuronal in origin.
- 25 193. The method of claim 192, wherein the non-neuronal cell is a COS-7 cell, a 293 human embryonic kidney cell, a LM(tk-) cell, a CHO cell, a mouse Y1 cell, or an NIH-3T3 cell.
- 30 194. A method of claim 118, wherein the cell is a mammalian cell.
- 35 195. The method of claim 194, wherein the mammalian cell is non-neuronal in origin.
196. The method of claim 195, wherein the non-neuronal cell is a COS-7 cell, a 293 human embryonic kidney cell, a LM(tk-) cell, a CHO cell, a mouse Y1 cell, or an NIH-3T3 cell.
197. A method of claim 178 or 180, wherein the lower urinary tract disorder is urinary incontinence or interstitial cystitis.
198. The method of claim 197, wherein the urinary

incontinence is urge incontinence.

199. The method of claim 197, wherein the urinary incontinence is stress incontinence.

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200. A method of treating urinary incontinence which comprises administering to a subject an amount of an antagonist of a human NPFF2 receptor effective to inhibit activation of the receptor and thereby treat incontinence.

10

201. The method of claim 200, wherein the urinary incontinence is urge incontinence.

15

202. A method of treating urinary retention which comprises administering to a subject an amount of an agonist of a human NPFF2 receptor effective to activate the receptor and thereby treat retention.

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203. A method of treating hypertension which comprises administering to a subject an amount of an antagonist of a human NPFF1 receptor effective to inhibit activation of the receptor and thereby treat hypertension.

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204. A method of treating hypotension which comprises administering to a subject an amount of an agonist of a human NPFF1 receptor effective to activate the receptor and thereby treat hypotension.

30

205. Use of a human NPFF2 receptor antagonist for the preparation of a pharmaceutical composition for treating urinary incontinence.

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206. The use of claim 205, wherein the urinary

incontinence is urge incontinence.

5 207. Use of a human NPFF2 receptor agonist for the preparation of a pharmaceutical composition for treating urinary retention.

10 208. Use of a human NPFF1 receptor antagonist for the preparation of a pharmaceutical composition for treating hypertension.

15 209. Use of a human NPFF1 receptor agonist for the preparation of a pharmaceutical composition for treating hypotension.

20 210. A method of modifying the feeding behavior of a subject which comprises administering the subject an amount of a compound which is a NPFF2 receptor agonist effective to decrease the consumption of food by the subject so as to thereby modify the feeding behavior of the subject.

25 211. The method of claim 210, wherein the subject is a vertebrate, a mammal, a human or a canine subject.

30 212. A method of treating a feeding disorder in a subject which comprises administering to the subject an amount of a compound which is NPFF2 receptor agonist effective to activate the receptor and thereby treat the feeding disorder.

35 213. The method of claim 212, wherein the feeding disorder is bulimia, bulimia nervosa or obesity.

214. The method of claim 212, wherein the subject is a vertebrate, a mammal, a human or a canine subject.

5 215. A method of inhibiting feeding which comprises administering to a subject an amount of an agonist of a human NPFF2 receptor effective to activate the receptor and thereby inhibit feeding.

10 216. Use of a human NPFF2 receptor agonist for the preparation of a pharmaceutical composition for inhibiting feeding.

15 217. Use of a human NPFF2 receptor agonist for the preparation of a pharmaceutical composition for treating a feeding disorder.

add B₁